

**APPLICATION
FOR
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**TITLE: METHOD FOR LOCALLY HIGHLY
RESOLVED, MASS-SPECTROSCOPIC
CHARACTERIZATION OF SURFACES USING
SCANNING PROBE TECHNOLOGY**

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Method for locally highly resolved, mass-spectroscopic
characterization of surfaces using scanning probe technology

The present invention relates to a method for high-resolution
5 microscopic observation of the surface structure, and at the same
time of the molecular composition associated with the observed
structure elements, of a sample surface. The invention also relates
to an apparatus having a specially adapted scanning force microscope
and a specially adapted mass spectrometer for carrying out the
10 method.

A scanning force microscope (SFM) scans the surface structure of a
sample to be examined, by means of a piezoelectric mechanism. The
mechanism can be moved not only on the sample plane (x/y direction)
15 but also at right angles to it in the z direction. First of all, the
sample is moved into contact with a tip by moving it in the z
direction. The tip is located at the free end of a cantilever which
is clamped in at one end. The cantilever typically has a length of
between 10 μm and 500 μm , and the tip is ideally atomically sharp.
20 The cantilever and tip are generally integrated and, in most
commercial products at the moment, are composed of silicon or
silicon nitride. The bending of the cantilever as a result of the
force between the sample and the tip is normally measured by means
of the optical pointer principle, and is set to a desired (nominal)
25 value.

In the so-called contact mode, an image of the sample surface is
obtained as follows: while a section of the sample surface is being
scanned, any further bending of the cantilever resulting from the
30 sample topology is fed back to the nominal value. The setting of the
scanning unit in the z direction as a function of each point on the
x/y plane reflects the sample topology, and is recorded.

In the so-called intermittent contact mode, the cantilever is caused
35 to oscillate close to its mechanical resonant frequency before being
moved towards the sample. After being moved towards the sample, the
tip then touches the sample briefly on one occasion in each
oscillation cycle. This leads to attenuation of the oscillation and

thus to a reduced oscillation amplitude, which is measured and is set to a specific value as a measure of the intensity of the interaction between the sample and the tip. The sample surface is now imaged as described above.

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Time-of-flight (TOF) mass spectroscopy is used to examine the molecular composition of an analyte on the basis of the molecular masses of the components. The elements of a sample to be examined are changed from the solid phase to the gas phase in different ways in a vacuum system. By way of example, one sample region is bombarded with a laser pulse for this purpose. In the process, charged molecules or molecule fragments which are accelerated by means of electrodes in a flight tube from which the air has been removed strike a detector after a flight path of, for example, 60 to 100 cm. The molecular weight is calculated from the time of flight: the heavier the molecule, the longer is the time of flight. This method is highly sensitive and accurate; only subpeco molar quantities are required. In principle, it is technically possible to detect individual ions in a TOF arrangement. The error is around \pm 0.05 Da per 1000 Da.

The primary aim of scanning force microscopy is to allow the state of the sample surface to be assessed by imaging the structure. If the preconditions are ideal, the atomic structure of a sample surface can be resolved. This applies to surface of crystalline structures and, to a restricted extent, to high-order organic and inorganic adsorbates on surfaces. In this situations, the state of the sample surface can be assessed directly.

However, depending on the sample, the resolution is generally not achieved, and the topography does not provide sufficient information to make an assessment of the state of a sample surface. In these situations, it is necessary to identify the local chemical nature or the local molecular composition of a sample surface by means other than microscopic structure analysis. This statement relates not only to scanning force microscopy but also to every other microscopic method (electron microscopy, optical microscopy, etc.). Methods are therefore used which combine microscopic imaging with chemical analysis, in the wide or narrow sense. The following text describes

two methods which are related to the method according to the method and are based on local ablation of the surface, followed by mass spectroscopy.

- 5 In laser desorption mass spectrometry (LAMMA), a laser pulse is focused onto a sample point chosen by means of conventional optical microscopy. This leads to local ablation of the sample and to the production of molecule ions from the locally ablated material. The ions are accelerated in the electrical field and are identified on the basis of their molecular mass by means of a time of flight mass spectrometer. One arrangement from LAMMA (LAMMA 2000; Spengler B. and Hubert, M.: Scanning Microprobe Matrix-Assisted Laser Desorption Ionization (SMALDI) Mass Spectrometry: Instrumentation for Sub-Micrometer Resolved LDI and MALDI Surface Analysis, J. Soc. Mass. Spectrom. 13, 735-748, 2002) should be mentioned in particular, in which the described principle has been optimized for combined imaging of the structure by means of confocal optical microscopy and local molecular composition by means of mass spectrometer of samples. In this arrangement, both the optical resolution and the minimum sample region from which ions can be obtained and detected are diffraction-limited. An optical and analytical resolution of 0.5 μm has been achieved in practice, that is to say the minimum analyzed sample region had a diameter of 0.5 μm .
- 25 Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is one analytical method for locally resolved chemical characteristic of material surfaces of an inorganic, organic and biological nature. The method is based on time-resolved recording of secondary ions which are produced by bombardment of the surface with high-energy primary ions (Cs^+ , Ga^+). In this case, the primary ion beam is highly focused and is scanned over the sample. The secondary ions released during the process are accelerated into the flight tube of a TOF mass spectrometer. Since the effective verification depth is only about 1 nanometer, the measured mass spectrum is composed only of the chemical components from the uppermost molecular layers. The lateral resolution of the ion images is about 1 micrometer.

The methods as described above for locally resolved chemical characterization of a sample surface vary with respect to the

minimum analyzed sample region within the resolution range of conventional optical microscopy. This is inadequate for many requirements in medicine, engineering and science. By way of example, cell membranes are laterally organized in a complex manner.

5 In this case, so-called lipid rafts represent the functional units of a large number of membrane-bound processes. Their diameter is about 60 nanometers. Analysis of their individual composition will be of critical importance for complete understanding of the membrane-bound processes that have been mentioned.

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The combination of structured imaging in the nanometer range with mass spectroscopy with corresponding position resolution promises an answer to the requirements that have been mentioned, and to a large number of other requirements. A combination of a scanning probe
15 technique (for example, SFM) with mass spectroscopy may be used for this purpose. In fact, so far, the option of combination of mass spectroscopy with high-resolution scanning force microscopy has been investigated in different ways by different authorities. Either sample material has been deliberately ablated by lateral injection
20 of pulsed laser light into the gap between the sample and the SFM tip, or pulsed laser light has been used to illuminate the sample, in the form of a pulse, through a glass fiber with a conical tip in a so-called aperture (SNOM (scanning near-field optical microscope)). Both strategies make use of the principle of near-field optics, that
25 is to say the tip is used to produce an illumination spot which is considerably smaller than the smallest possible diameter of an illumination spot produced by conventional optics.

This has made it possible to reproducibly produce holes with a
30 diameter of a few nanometers. In both cases, ions that were produced were sucked out laterally from the tip region. However, surprisingly, it has not so far been possible to achieve an unambiguous association between ions and a defined region in the near field of the tip. This problem is a result of the ions being
35 sucked out inefficiently from the near field of the tip and sample. Our own experimental investigations and model calculations have confirmed the unsatisfactory finding: the transmission of ions that are produced into the flight tube of a mass spectrometer is poor and is dependent in a manner which cannot be calculated on the geometric

conditions immediately at the point at which the ions are produced.

In summary, universal chemical analysis of surfaces with a position resolution in the nanometer range has not yet been available.

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The object of the invention is to specify an improved method and an improved apparatus in which ions are produced in a very small volume in the near field of a tip/sample region which can be selected by the scanning force microscope, and are passed on with a high
10 transmission level for mass spectrometry.

According to the invention, the object is achieved by an apparatus as claimed in the independent claim 1, and by a method as claimed in the independent claim 7.

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According to one aspect, a method is provided in which a scanning force microscope is operated with a cantilever with an integrated hollow tip. The hollow tip has a small aperture opening on the sample side. The aperture preferably has a diameter which is
20 considerably smaller than the wavelength of the light that is used. An illumination spot is thus produced on the sample on the basis of the principle of near-field optics, with a diameter which is considerably less than the diffraction-limited illumination spot of conventional optics. The hole in the tip widens increasingly towards
25 the rear side, where it has its largest opening.

In particular, the method (i) allows an unambiguous association between all the observed ions and a defined sample region; (ii) association of the observed ions with the sample topology; and (iii)
30 local resolution, both of the topology and of the local molecular composition, below the resolution limit of conventional optical systems.

Scanning force microscopy is preferably carried out conventionally,
35 as described above, using the intermittent contact mode or the contact mode. The microscopy is preferably carried out in a hard vacuum. As an alternative to scanning force microscopy, it is also feasible to adapt other scanning probe techniques for use in the described method.

The locally resolved mass spectroscopy is carried out in parallel with or following the SFM imaging. In this case, the tip is in contact with the sample, or is in the immediate near field of the sample. For mass spectroscopy, a laser pulse is injected into the hollow tip from the rear side. Material is ablated from the sample at each desired point on the sample by means of a short laser pulse, and is passed on for mass spectroscopy. For this purpose, the opening in the rear of the tip is actually connected to a flight tube, which is at a suitable electrical potential relative to the tip and to the sample, and is used for electrically sucking out the molecular ions which are produced after a laser pulse. The ions then preferably fly into a time of flight mass spectroscopy. Further advantages and expedient developments of the invention will become evident from the following description of exemplary embodiments and with reference to the drawing, in which:

Figure 1A shows a schematic illustration in order to explain a method based on one embodiment, showing a cross section through an arrangement with a scanning unit, a sample, a cantilever with a hollow tip, a flight tube and an objective;

Figure 1B shows an enlarged illustration of a section of an arrangement shown in Figure 1A;

Figure 2A shows a schematic illustration in order to explain a method based on a further embodiment, showing a cross section through an arrangement with a scanning unit, a sample, a cantilever with a hollow tip, a flight tube and an objective; and

Figure 2B shows an enlarged illustration of a section of the arrangement shown in Figure 2A.

Figures 1A, 1B, 2A and 2B show schematic illustrations in order to explain two embodiments of the method for locally high-resolution, mass-spectroscopic characterization of surfaces by means of a scanning probe technique. In both embodiments, scanning force micro-

scopy is combined with the capability to ablate surface material from the sample at any point x, y, and carry out mass-spectroscopic analysis of ionized sample material. This is done by using a cantilever 1 with a tip 2 with an axial, conical hole through it.

5 The hole opens with a defined aperture at the apex 3 of the tip.

The aperture is used as an outlet opening for a focused laser pulse 10 onto the sample 30, and as an inlet opening for molecular ions 20 which are produced after a laser pulse in the area of the illuminated sample region.

The sample is generally illuminated coaxially with respect to the longitudinal axis of the tip, through the hole in it. The ions are preferably likewise extracted coaxially with respect to the tip, and 15 through the hole. For extraction, the flight tube 21 is connected to an electrical potential relative to the sample. An electrical field is formed, largely axially symmetrically with respect to the flight tube/tip axis. The field penetrates the hole in the tip and leads to extraction of the ions. If the flight tube is at a relatively low 20 potential, ions with a positive total charge are extracted, and vice versa. The high degree of axial symmetry of the arrangement and thus of the field leads to largely axial extraction and to axial flight of the ions. Additional ion optics in the flight tube (not illustrated) are used to pass back ions which do not fly exactly 25 axially.

The area from which the material is ablated is governed by the size of the aperture in the hollow tip. The aperture diameter is typically considerably less than the wavelength of the light that is 30 used.

The embodiments in Figures 1A, 1B and 2A, 2B differ in how the laser light is injected: in the embodiment shown in Figures 1A and 1B, an objective 11 is located at the side, alongside the flight tube, for 35 focusing. The optical axis 12 is initially at right angles to the axis of the flight tube 21. The light enters the flight tube via a window, is deflected in the axial direction by means of a mirror 13, and is focused in the hole in the tip. The mirror has a central hole 24 for the ions to pass through.

In the embodiment shown in Figures 2A and 2B, the objective is coaxial with respect to the flight tube. The flight tube is introduced into a central hole 24 in the objective. Collimated laser
5 light is reflected into the beam path downstream from the objective. In this case as well, the mirror has a central hole for the ions to pass through.

The features of the invention as disclosed in the present
10 description, in the claims and in the drawing may be significant to the implementation of the various embodiments of the invention both individually and in any given combination.